UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 23

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum

Subject: Review of Ecotoxicity Data Submitted in Compliance with the Methoprene RED (DP Barcode D226999, Case No. 003099, MRIDs 440221-01 and 440221-02)

From: Mark J. Perry, Biologist
Biopesticides and Pollution Prevention Division (7501W)

Thru: J. Thomas McClintock, Team Leader
Biopesticides and Pollution Prevention Division (7501W)

To: Willie Nelson, Regulatory Action Leader
Biopesticides and Pollution Prevention Division (7501W)

Action Requested

Sandoz Agro, Inc. submitted a chronic toxicity study performed with mysid shrimp (guideline reference 72-4) and a study evaluating non-target effects in metropolitan wetland areas (non-guideline). Both studies were performed with technical methoprene and were submitted in response to the methoprene RED. The non-guideline study was required by the Agency prior to reclassification of methoprene as a biochemical. This study also evaluated the use of *B. thuringiensis israelensis* as a mosquito larvicide.

Results/Conclusion

The non-target effects in metropolitan wetland areas (non-guideline) study is classified supplemental; it was not conducted following GLP regulations 40 CFR 160. Although the study may provide useful information, it does not satisfy the data requirement. In general, the results of the study indicate that, with field use of methoprene in wetlands, adverse ecological effects on non-target organisms were either not present or were unmeasurable due to natural variability or study design constraints.

The chronic toxicity (72-4) study was conducted according to acceptable procedures and determined the following values for methoprene technical to mysid shrimp (*Mysidopsis bahia*): LOEC of 25 μg a.i./L, NOEC of 14 μg a.i./L, and MATC >14 and <25 μg a.i./L (geometric mean MATC = 19 μg a.i./L). This study was adequately conducted (core) and provides acceptable data. Although the results are valid, the expected effect on aquatic invertebrates cannot be evaluated without the estimated environmental concentration (EEC) determined from the recommended use levels for this product.

DATA EVALUATION REPORT

METHOPRENE

STUDY TYPE: NON-GUIDELINE STUDY

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
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Oak Ridge National Laboratory
Oak Ridge, TN 37831

Primary Reviewer:
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Signature: Sylvia S. Talmage
Date: October 2, 1996

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Robert H. Ross, M.S., Group Leader

Signature: RHR
Date: 10-3-96

Paul G. Forsyth, Ph.D.

Signature: for Paul Forsyth
Date: 10-3-96

Quality Assurance:
Susan Chang, M.S.

Signature: Susan Chang
Date: 10/3/96

Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

METHOPRENE

Non-Guideline Study

EPA Reviewer: Mark J. Perry
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division

Date: 10-22-96

Date: 10/23/96

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 44022102, An Assessment of the Non-Target Effects of the Mosquito Larvicides, Bti and Methoprene, in Metropolitan Area Wetlands

DB BARCODE: D226999
CASE: 003099
REG./FILE#: 002724-00375
CHEMICAL/BIOL#: 105401 Methoprene

COMPANY/SPONSOR: Sandoz Agro, Inc.

TEST MATERIAL: Methoprene

REVIEW CONCLUSION: This non-guideline study is classified supplementary; it was not conducted following GLP regulations 40 CFR 160. The original data, particularly for the Wright County Long Term Experiment, should be provided in order to perform a more definitive evaluation. In general, the results of the studies indicated that, with field use of methoprene in wetlands, adverse ecological effects on non-target organisms were either not present or were unmeasurable due to natural variability or study design constraints.

RECOMMENDATIONS: The sponsor should provide copies of the original unpublished laboratory and field studies for review. The Wright County Long Term Experiment could serve as a Tier IV Simulated or Actual Field Testing for Aquatic Organisms (Guideline M 154A-34) if the entire report including raw data were submitted. A combination of the Wright County Long Term Experiment and the Wright County Historical Survey could serve as a Tier IV Simulated or Actual Field Testing for Birds (Subdivision M, Guideline 154A-33) if the entire report including raw data were submitted.

ADEQUACY OF STUDY: Supplementary; this study was not intended to fulfill a guideline requirement but was intended to support reregistration of methoprene.

MATERIALS & METHODS: The study summarizes five field studies in which the effects of the application of methoprene to Michigan wetlands on non-target organisms was evaluated. The individual studies were suggested and sponsored by the Scientific Peer Review Panel of the Metropolitan Mosquito Control District and addressed the long-term ecological effects of a larvicidal program. As such, these studies did not follow the principles of GLP as outlined in 40 CFR Part 160. Additional methodology is summarized under the discussion of the individual studies.

REPORTED RESULTS: The results were provided as summaries of the five individual studies.

1. In the Wright County Historical Survey, no statistical differences in growth, reproduction, or return rates of red-winged blackbird populations or species composition and density of invertebrates were found between wetlands treated with methoprene and untreated sites.

2. In the North Metropolitan Area Bird Survey, observed differences in bird populations between untreated wetlands and wetlands historically treated with either methoprene or another larvicide were not clearly treatment related. No distinction between methoprene-treated sites and sites treated with the other larvicide was made.

3. In the Lake Maria Study, no statistically significant changes in densities of zooplankton, insects, or benthic invertebrates were observed between the methoprene-treated and untreated areas of Lake Maria. However, densities were too low for the statistical evaluation to be rigorous.

4. In the Mallard Duckling Study, differences in duckling weight were observed after five days (first trial) between treated and untreated sides of three ponds but not after 30 days (second trial). Methoprene treatment did not change the abundance of aquatic insects compared to untreated parts of the ponds.

5. Results of the Wright County Long Term Experiment indicate that following three years of application of methoprene to Michigan wetlands, there were no significant effects on zooplankton or bird populations. Significant reductions in benthic invertebrates were limited to chironomids (midges) which are closely related to mosquitoes.

DISCUSSION: The synthetic insect growth regulator methoprene has been studied for over 20 years. Review of the published and unpublished literature and the summary of information submitted by the registration applicant indicate that the only ecological effect of concern, as indicated by laboratory toxicity studies, is reduced reproduction of some non-target invertebrates such as *Daphnia* sp. Results of multi-year field studies in which methoprene was applied to Michigan wetlands as well as two historical studies involving the comparison of previously treated and untreated wetlands in the state of Michigan showed that zooplankton and avian reproduction and density were not affected by methoprene treatment; in one study, densities of some aquatic insects (chironomids and other benthic flies) were reduced by methoprene treatment. However, natural variability, the length of time over which the studies were conducted, and the response at other sites make the results of the field studies difficult to interpret.

These studies were not conducted according to prescribed procedures and should be considered supplementary. Studies address the ecological consequences of a long-term larvicidal program. Although natural variability occurs among sites and confounding factors such as fluctuating water levels were present, the Wright County Long Term Experiment indicates that, with the exception of reduced numbers of midges, there were no observable adverse ecological effects within the three-year treatment period. More precisely, there were no statistically significant decreases in cladoceran (which had been identified as sensitive non-target organisms) density or species richness between treated and untreated sites over the three-year treatment period. It is the opinion of the Scientific Peer Review Panel and the reviewer that the Wright County Long Term Experiment study should be continued for several more years.

The published literature indicate that methoprene is not persistent in the environment; however, application of slow release formulations or briquets ensure its presence over time. Because analysis of natural waters for methoprene is difficult due to interfering substances, some effort to measure concentrations in containers held under natural environmental conditions should be made.

The published literature also indicate that methoprene is practically nontoxic to mammals and birds and is not a reproductive toxicant. In addition, metabolism in a variety of species has been demonstrated. Therefore, the lack of effects on avian populations at the studied sites is not unexpected.

Although the Wright County Long Term Experiment was well summarized and data were provided, the original reports are necessary to perform a definitive evaluation.

DISCUSSION OF INDIVIDUAL STUDIES:

1. **Wright County Historical Survey**

Method: The purpose of this study was to compare 10 wetlands in the state of Michigan that had been treated with methoprene for two or more consecutive years with 30 wetlands outside the boundary of treatment. Comparisons were made in terms of effects on growth and reproduction of nesting red-winged blackbirds, (during one year) the yearly return rate of male red-winged blackbirds (two-year study), and on zooplankton and benthic invertebrates (one-year study). Zooplankton were collected with funnel-traps and benthic aquatic invertebrates were sampled with benthic cores.

Results: No differences between treated and untreated sites were detected in average clutch size, egg size, nestling growth rates, fledgling mass or fledgling ages of red-winged blackbirds. "Reproductive success was highly variable among sites, but appeared to be lower at sites where marsh wrens and yellow-headed blackbirds were present." Return rates of males were lower in the two years of study, but could not be correlated with an effect on the food web as determined by territory size, harem size, and egg and nest survival probabilities. No statistical differences were found between the treated and untreated sites for red-winged blackbird populations ($p=0.05$) or invertebrate populations ($p=0.05$); the raw data were not provided.

Discussion: Natural variability inherently makes comparisons among sites difficult in field studies. As noted by the authors, drought during the study year had lowered water levels, eliminating some areas and reducing densities of invertebrates in others with the result that treatment was difficult to distinguish from natural variation. The authors also noted that the treated sites had not been treated for very many consecutive years and the number of treatments per year in preceding years was relatively low. This study can be considered preliminary rather than definitive.

2. **North Metropolitan Area Bird Survey**

Method: Terrestrial breeding birds in treated and untreated wetlands in three counties were censused. Eleven sites historically treated with methoprene and 23 sites historically treated with *Bacillus thuringiensis israelensis* (Bti) were paired with untreated sites on the basis of their area, shape, vegetation, and water regimes. Sites were selected using a double-blind approach. Bird populations were surveyed twice (mid-May to early July) using the variable circular plot technique. Nests of tree swallows in wooden nest boxes were monitored in seven matched pairs of sites during three years to estimate occupancy rates, clutch size, egg success, nestling growth rates, and fledgling success. The authors did not distinguish between methoprene and Bti-treated sites. Raw data were not provided.

Results: Of 26 different species of birds, only densities of yellow-headed blackbirds was significantly lower on the treated wetlands and their densities were negatively correlated with number of years of previous treatment. Growth of tree swallow nestlings was slightly retarded in treated wetlands during the first study year with nestlings from treated wetlands fledging about 2 days later,

but at approximately the same mass as those in non-treated wetlands; differences in fledgling age were not detected in the second and third years of the study.

Discussion: The study is not useful for ascertaining the effects of methoprene on bird populations as the investigators did not distinguish between methoprene and Bti-treated sites. In addition, as noted by the authors, many of the species censused are only weakly dependent on wetlands, effects on tree swallow fledgling growth were variable from year to year, and the small number of sites limited the power of the study to detect small effects of treatment. The study is not useful for ascertaining the effects of methoprene on bird populations.

3. Lake Maria Study

Method: Two wetland areas were trisected radially with curtains of polyolefin material. In April, one sector of a wetland was treated with a 150-day methoprene briquet (water concentration not stated/measured) and the other two sectors were treated with placebos. All sectors of the other wetland area received placebos. The different areas were sampled (time not stated) for zooplankton with funnel traps and for benthic invertebrates with benthic cores. A pre-treatment census was not mentioned.

Results: No statistically significant changes in densities of zooplankton, insects, or benthic invertebrates were observed between the treated and untreated sites.

Discussion: Few details of the study were provided. Aquatic organisms were not identified, but it can be assumed that they were similar to those in the accompanying studies. It appears that only one area was treated, although untreated areas were part of the same wetland. If present, larvicidal action should have been observable; however, it was noted by the authors that densities of the orgnaisms of concern, benthic invertebrates, were too low to provide a rigorous test of the action of the larvicide. It was also stated that the dosage of methoprene was high enough to cause effects, but dosage was not stated. The study can be considered supplementary.

4. Mallard Duckling Study

Methods: Three ponds were bisected with double plastic barriers; randomly selected halves were treated with either methoprene briquets or placebos. Broods of 10 human-imprinted ducklings were placed in each wetland half and growth was observed for 5 (first trial) or 31 days (second trial) after initiation of treatment. Briquets stranded by receding water levels were replaced. Benthic organisms (food for the foraging ducklings) were sampled prior to and post-treatment. Floating traps were used to sample emerging insects.

Results: In the first trial ducklings from the treated site weighted less after 5 days of foraging than ducklings from the untreated site (no data provided); in the second trial, there was no difference in weights of ducklings between the treated and untreated halves. No significant differences in the density of benthic larvae or emerging adults were found between the sites. Data were not provided.

Discussion: No conclusions can be drawn from this study as weight differences of ducklings observed in the first trial were not evident in the second, longer trial. Treatment in the first trial was too short to affect insect densities and treatment during the second trial did not change the abundance of insects. Methoprene concentrations were not measured.

5. Wright County Long Term Experiment (WCLTE)

Method: This is a 5-year study (2-years pre-treatment and 3 years of treatment) of 17 wetland sites (9 reference sites and 8 methoprene treatment sites) in Wright County, Michigan. Six applications/year during spring and summer at rates ranging from 1.1 to 13.2 lbs/acre were made; the material was in the form of a 20-day release granule formulation. Treatments were monitored with bucket samplers placed in each wetland to measure the amount of material that was applied. Monitoring also included emergence success of mosquito larvae collected from treated and untreated sites. In addition to sampling for mosquitoes, populations of zooplankton and benthic invertebrates were sampled at 3-4 week intervals during the spring and summer of each year. Results from treatment sites were compared with reference sites using an ANOVA in three ways: date by date within each year, on a yearly basis across dates within each year, and averaged over the three treatment years ($\alpha = 0.05$). Breeding birds were censused and blackbirds were examined for reproduction and behavior. Data were provided in graphs and tables.

Results: The presence of methoprene at the sites was indicated by the reduction in emergence of mosquito larvae during the last two years of the study. In 1992 emergences of collected larvae were 72% at the reference sites and 17% at the treated sites; the respective values in 1993 were 70% and 10%.

No effects on zooplankton occurred over the three years as indicated by species diversity, density, size, or reproduction. Although no effects on benthic invertebrates were detected during the first year of treatment, density and biomass were reduced compared with the control sites during the second and third year. Decreases at the treated sites were primarily due to reduced populations of chironomid larvae (midges) and other primitive flies. Midges were the most abundant and diverse group of benthic invertebrates at the sites.

Censuses of 19 breeding bird populations and a detailed study of red-winged blackbirds showed no consistent changes during the years of study. The censuses included three species that feed primarily on aquatic insects (soras, Virginia rails, and marsh wrens).

Discussion: Reduced densities of aquatic insects, particularly midges, which are closely related to mosquitoes, would be expected. Although the larvicidal program is aimed at mosquito control, the control of midges might not be considered detrimental to the environment unless some species of wetland birds are dependent on midges as their major food source. There were no declines in cladocerans which had been identified as sensitive non-target organism.

The red-winged blackbird is not dependent on wetlands for habitat and food but was the most abundant species and adequate for sampling. If possible, reproduction and development of the most abundant species of wetland species that feeds primarily on aquatic insects should be studied.

DATA EVALUATION REPORT

(S)-METHOPRENE TECHNICAL

STUDY TYPE: LIFE-CYCLE - MYSID SHRIMP (72-4)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Date: 10-3-96

Sylvia S. Talmage, Ph.D., D.A.B.T.

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Date: October 5, 1996

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Date: 10/3/96

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

(S)-METHOPRENE TECHNICAL | Mysid Life-Cycle Study (72-4)

EPA Reviewer: Mark J. Perry — Date: 10-22-96
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D. — Date: _____
Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 44022101, (S)-Methoprene Technical - Chronic Toxicity to Mysids (*Mysidopsis bahia*) Under Flow-Through Conditions

DP BARCODE: D226999 **CASE**: 003099
REG./FILE#: 002724-00375 **CHEMICAL/BIOL#**: 105401 Methoprene

COMPANY/SPONSOR: Sandoz Agro, Inc., 1300 E. Touhy Avenue, Des Plaines, Illinois 60018

TEST MATERIAL: (S)-Methoprene Technical

REVIEW CONCLUSION: This study was conducted according to acceptable procedures and determined the following values for (S)-methoprene technical to mysid shrimp: LOEC of 25 μg a.i./L, NOEC of 14 μg a.i./L, and MATC >14 and <25 μg a.i./L (geometric mean MATC = 19 μg a.i./L). This study was adequately conducted and provided useful data.

RECOMMENDATIONS: None

ADEQUACY OF STUDY: Core

MATERIALS & METHODS: The study procedures followed those of the Springborn Laboratories, Inc. (Wareham, MA) protocol entitled "(S)-Methoprene - Life-Cycle Toxicity Test with Mysids (*Mysidopsis bahia*), Following FIFRA Guideline 72-4" (Springborn Laboratories Protocol #:081295/FIFRA/530/s-methoprene [1995] and Protocol Amendment #1 [1995]). The study was conducted in accordance with GLP 40 CFR 160 with the exception of routine water screening and food analyses for pesticides, PCB's, and toxic metals. The water screening and food analyses were conducted using standard U.S. EPA procedures by Lancaster Laboratories (Lancaster, PA). No protocol deviations were noted and the study was acceptably conducted. The test material, (S)-Methoprene technical (Lot No. 5S1008, CAS# 40596-69-8), was received from Sandoz Agro, Inc. (Dallas, TX) and was stored frozen. The test material was an amber liquid with a purity of 95.311%, molecular weight of 310.5 g/mol, water solubility of 0.52 ppm, and vapor pressure of <1 mm Hg. An analytical standard of (S)-Methoprene (Lot No. 95-24), was received from the same source and was an amber liquid with a purity of 95.21 $\pm$ 0.01%. The analytical standard was also stored frozen.

The mysids ($\leq$24 hours old) used in these tests were obtained from laboratory cultures maintained at Springborn Laboratories (SLI Lot #95A107) and were kept in recirculated, filtered artificial seawater for 14 days prior to the test. Juvenile mysids ($\leq$24 hours old) were collected and fed brine shrimp (*Artemia salina*) nauplii, *ad libitum*, twice daily, with one feeding supplemented with Selco®, a liquid food supplement. Food sources were analyzed routinely and found to be acceptably free of pesticides, PCB's, and metals considered toxic to mysids.

Artificial seawater used as dilution water during these tests was prepared by the addition of a commercially prepared salt formula (hw-MARINEMIX®) to filtered soft freshwater having a hardness of 20 to 40 mg/L as $CaCO_3$, with a final salinity of 25 ± 3‰. The prepared dilution was aerated vigorously for approximately 24 hours, then allowed to aerate for an additional 24 hours prior to use. Routine analyses found no toxic concentrations of pesticides, PCBs, or toxic metals in the dilution water source. Mysids maintained in artificial seawater prepared from the same source as the artificial seawater used in this study have successfully survived and reproduced over several generations.

Nominal concentrations selected for the test material were 9.4, 19, 37, 75, and 150 µg a.i./L. A 30 mg a.i./mL stock solution was prepared by dissolving 1.584 g of test material with acetone to volume in a 50 mL volumetric flask. Additionally, a 0.50 mL/mL solvent stock solution was prepared by diluting 50 mL of acetone with distilled water to volume in a 100 mL volumetric flask.

The life-cycle test was conducted using an exposure system consisting of a constant-flow serial diluter, a temperature-controlled water bath, and a set of 14 exposure aquaria (two per test concentration level). Each aquarium contained two mysid retention chambers made of glass Petri dishes covered with screen which were used to maintain non-paired mysids during the study. Pairing chambers, used to house sexually mature male and female organisms, were cylindrical glass jars having two screen-covered holes. The aquaria systems allowed for adequate solution exchange via siphon drains. The 150 µg a.i./L nominal treatment was attained by delivering 0.0015 mL/min of the test material stock solution to a mixing chamber which also received 0.302 L/min of dilution water. The stock solution was proportionally diluted (50% dilution factor) to provide the remaining nominal test concentrations. A similar system was used to deliver the acetone stock solution to the diluter system of the solvent test chambers, providing an acetone concentration equivalent to the acetone concentration in the highest test solution. The solution exchange system operated at a rate of approximately 15 aquarium volume additions per day to provide a 90% test solution replacement rate of approximately 3.5 hours. The entire operating system was illuminated with fluorescent lighting for 16 hours daily followed by 8 hours darkness.

"Mysids, ≤24 hours old, were collected form the Springborn culture unit and divided among 28 beakers. The beakers contained culture water and were held in a waterbath maintained at 25 ± 2°C. The organisms were impartially selected and distributed to the beakers by adding five organisms at a time to each beaker until each beaker contained 15 mysids. Each group of 15 mysids was then transferred to one of the 28 labeled retention chambers (two per aquarium). The test was initiated when the retention chambers were placed in their respective test aquaria. Each test aquarium contained two retention chambers, yielding 30 mysids per replicate vessel and 60 organisms for each treatment level and control."

Upon reaching sexual maturity (Day 15), mature male/female pairs within each exposure aquarium were transferred from the retention chambers to the 10 glass pairing jars (one pair per jar). The remaining mysids were all placed in one of the initial retention chambers within each aquarium and maintained for the duration of the chronic test. Male mysids from this pool were used to replace dead males removed from the paired groups. Females that died in pairing jars were not replaced. If development of brood pouches, distinguishing females from males, was delayed due to toxicant exposure, all test organisms were maintained in the retention chambers until maturity was observed or until test termination. Mysids were fed live brine shrimp (*Artemia salina*) nauplii twice daily. Before pairing, at least one of the daily feedings was enriched with Selco®. After pairing, the mysids were fed Selco®-enriched brine shrimp nauplii once every other day.

During the first 14 days, observations were made for mortality and any abnormal appearance or behavior. After pairing (Day 15), mortality of the paired mysids, the number of offspring produced by each female, and any abnormal appearance or behavior was recorded. Observations were made daily throughout the study. Dead mysids were removed and discarded.

At test termination, all mysids were sacrificed and measured for individual body length (nearest 0.1 mm) and total dry body weight (nearest 0.01 mg). Reproductive success was calculated for each replicate aquarium as the ratio of the total number of offspring produced to the total number of females contained within each chamber per reproductive day. The number of female reproductive days was determined as the number of days that an individual was alive, counting the day that offspring were first observed in any control (i.e., Day 18 represents reproductive day 1).

Daily measurements were made for water temperature, dissolved oxygen concentration, pH, and salinity in each replicate of each treatment. Samples were removed from each replicate test solution and control on days 0, 7, 14, 21, and 28 and analyzed for test material concentration.

Data from the paired and unpaired mysids were statistically analyzed for treatment effects. Endpoints analyzed for first generation (F_0) mysids included survival, growth (i.e., body weights and lengths), and reproduction. Reproductive success was determined only for the paired organisms. Bartlett's Test was used to test for homogeneity of variance (99% certainty level). Student's t-test was conducted for each endpoint to compare solvent and negative controls, resulting in no significant difference. Therefore, solvent and negative control endpoints were pooled for the remaining comparisons between controls and treatments. The Williams Test was used to determine treatment level effects (95% certainty level). The Maximum-Acceptable-Toxicant-Concentration (MATC), or the theoretical threshold concentration of the test material expected to produce no deleterious effects to mysids, was estimated at the 95% certainty level. Also determined were the Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC).

REPORTED RESULTS: Water quality parameters measured during the 28-day exposure remained within acceptable limits. Analyses of test material concentrations in the aquaria exhibited consistency between replicates and sampling intervals and the expected concentration gradient across treatment levels was maintained throughout the 28-day test. However, mean measured concentrations ranged from 66 to 77% of the nominal concentrations and defined the concentrations tested as 7.2, 14, 25, 50, and 98 μg a.i./L. Coefficients of variation averaged 15% for all mean measured concentrations.

Survivals of the F_0 mysids were 90 and 92% for the control and solvent control, respectively, with no statistical difference between the two (pooled control survival = 91%). Survivals of 78, 78, 82, 83, and 57% were observed for mysids exposed to mean measured test material levels of 7.2, 14, 25, 50, and 98 μg a.i./L, respectively. Only the 98 μg a.i./L concentration was determined to be statistically different from the pooled control results. For this reason, results for that treatment were eliminated from further chronic statistical analyses.

No statistical difference was observed between control and solvent control mysids for reproductive success (0.6 and 0.39 offspring/female/reproductive day, respectively) and these groups were pooled (mean = 0.50 offspring/female/reproductive day). Mysid reproduction in the treatment levels that did not adversely affect survival, i.e., 7.2, 14, 25, and 50 μg a.i./L, ranged from 0.22 to 0.45 offspring/female/reproductive day and were determined not to be significantly different from the pooled control organisms with respect to reproductive success.

The mean body lengths of male and female control mysids were 7.0 and 6.9 mm, respectively, while the solvent control mysids measured 7.2 and 7.0 mm for males and females, respectively. The control and solvent control body length measurements were not statistically different, and the pooled lengths for control males and females were 7.1 and 7.0, respectively. For exposure concentrations to the test material of 7.2, 14, 25, and 50 μg a.i./L, the respective body lengths for male mysids were 7.1, 7.2, 7.2 and 7.1 mm, while the respective body lengths for females were 7.2, 7.1, 7.2, and 6.9 mm. Both male and female body lengths were not statistically different from the pooled control body lengths. These data indicate that the test material "at levels $\leq$ 5.0 μg a.i./L" did not adversely affect organism growth based on body length. Obviously, this should read "at levels $\leq$ 50 μg a.i./L".

The mean body weights for the control and solvent control male mysids were 0.88 and 0.82 mg, respectively, while those for females were 1.0 and 0.90 mg, respectively. There were no statistical differences between control and solvent control groups for either males or females, allowing for pooled averages of 0.85 and 0.95 mg for males and females, respectively. For exposure concentrations to the test material of 7.2, 14, 25, and 50 μg a.i./L, the respective dry body weights for male mysids were 0.78, 0.82, 0.75, and 0.78 mg, while respective dry body weights for females were 0.93, 0.93, 0.93, and 0.81 mg. Statistically significant reduced dry body weights occurred in exposure concentrations to the test material of 25 and 50 μg a.i./L for males and 50 μg a.i./L for females.

"Based on the results of this study, the LOEC and NOEC of (S)-Methoprene technical for mysid survival, reproductive success and growth (total body length and dry weight) was determined. Dry body weight of male mysids was determined to be the most sensitive indicator of toxicity of (S)-Methoprene technical to mysids. The LOEC and NOEC, based on male dry body weight, was 25 and 14 μg a.i./L, respectively. The Maximum-Acceptable-Toxicant Concentration (MATC) was calculated to be $>$14 and $<$25 μg a.i./L (Geometric Mean, MATC = 19 μg a.i./L). These data provided a MATC which corroborated the conservatively estimated MATC (i.e., 24 μg a.i./L) determined during previously conducted life-cycle tests (SLI Report #92-11-4518)."

DISCUSSION: This study was conducted following acceptable procedures outlined in FIFRA Guideline 72-4, Subdivision E of the U.S. EPA Pesticide Assessment Guidelines (1982). This study determined the following values for (S)-methoprene technical to mysid shrimp: LOEC of 25 μg a.i./L, NOEC of 14 μg a.i./L, and MATC $>$14 and $<$25 μg a.i./L (geometric mean MATC = 19 μg a.i./L). These values are based on the dry body weight for male mysids, which was determined to be the most sensitive performance criterion measured in these tests. The mortality data (presented as "Percent Survival") were reported to be significant only at the 98 μg a.i./L level, and sublethal data at this level were not used in statistical calculations.

Although mortality was measured, no LC_{50} was calculated since 50% mortality was never achieved, nor did the data seem to follow a dose-response curve, i.e., percent survival was lower at the two lower treatment concentrations (78% for both) than at the next two higher treatment concentrations (82 and 83%) but lowest at the highest concentration of 98 μg a.i./L (57%). The survival data shown in Table 1 are the actual percentages measured in each aquarium, with the mean given for the two aquaria per concentration. The reduction in survival does not follow a dose-response fashion, except that the greatest mortality occurs in the highest treatment concentration level. Although the Williams' Test showed no significant difference in survival between each of the treatments and the pooled controls (at $\leq$50 μg a.i./L), the use of only two data points (per treatment) does not give a standard deviation and is of questionable statistical validity.

The authors report no significant effect of the test material on reproductive success in mysids. However, the reproductive data shown in Table 1 is presented in a similar fashion to the survival data. When the number of reproducing females and the number of reproductive days are divided out, all of the reproductive data within an aquarium is reduced to a single number. Again, the use of only two values is of questionable statistical validity. The reviewer repeated the statistical analyses of the author regarding survival and reproduction and concurs with the author's conclusion.

TABLE 1. Summary of the first generation (F_0) survival and reproductive success (offspring/female/reproductive day) during the 28-day life-cycle exposure of mysids (*Mysidopsis bahia*) to (S)-Methoprene Technical

Mean Measured Concentration µg a.i./L	Replicate	Percent Survival[a]	Reproductive Success[a]
Control	A	90	0.41
	B	90	0.79
	Mean	90	0.60
Solvent Control	A	90	0.33
	B	93	0.44
	Mean	92	0.39
Pooled Control[b]	Mean	91	0.50
7.2	A	73	0.42
	B	83	0.29
	Mean	78	0.36
14	A	73	0.44
	B	83	0.46
	Mean	78	0.45
25	A	87	0.49
	B	77	0.25
	Mean	82	0.37
50	A	83	0.18
	B	83	0.25
	Mean	83	0.22
98	A	60	0.083
	B	53	0.0094
	Mean	57[c]	0.046[d]

Data taken from Table 3, p. 34, MRID 44022101.

[a] Values presented have been rounded to two significant figures.

[b] Since control and solvent control data were not determined to be significantly different, all treatment data were compared to the pooled control data.

[c] Significantly different ($p \leq 0.05$) from the pooled control (Williams' Test).

[d] Since organism survival was adversely affected, this treatment level was excluded from statistical analysis to determine treatment effects for body length, body weight, and reproductive success.

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